



10025222.060302 #7

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN
Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 1 of 24

SEQ ID NO: 1

>STAAU_R009 nucleotide sequence: 1800
TTGCGAATAGATCAATCGATCATTAAATGAAATAAAAGATAAAACCGACATTTTAGACTTGGTAAGTGA
ATATGTAAAATTAGAAAAGAGAGGACGCAATTATATAGGTTTGTGTCCTTTTCATGATGAAAAGACAC
CTTCATTTACAGTTTCTGAAGATAAACAAATTTGTCAATTGTTTTGGTTGTAAAAAAGGTGGCAATGTT
TTTCAATTTACTCAAGAAATTAAAGACATATCATTGTTGAAGCGGTTAAAGAATTAGGTGATAGAGT
TAATGTTGCTGTAGATATTGAGGCAACACAATCTAACTCAAATGTTCAAATTGCTTCTGATGATTTAC
AAATGATTGAAATGCATGAGTTAATACAAGAATTTTATTATTACGCTTTAACAAGACAGTCGAAGGC
GAACAAGCATTAAACGTACTTACAAGAACGTGGTTTTACAGATGCGCTTATTAAAGAGCGAGGCATTGG
CTTTGCACCCGATAGCTCACATTTTTGTGTCATGATTTTCTTCAAAAAAAGGGTTACGATATTGAATTAG
CATATGAAGCCGGATTATTATCACGTAACGAAGAAAATTTTCAGTTATTACGATAGATTTTCGAAATCGT
ATTATGTTTCCTTTGAAAAATGCGCAAGGAAGAATTGTTGGATATTCAGGTCGAACATATACCGGTCA
AGAACCAAAATACTTAAATAGTCCTGAAACACCTATCTTTCAAAAAAGAAAGTTGTTATACAACCTTAG
ATAAAGCGCGTAAATCAATTAGAAAATTAGATGAAATCGTATTACTAGAAGGTTTTATGGATGTTATA
AAATCTGATACTGCTGGCTTGAAAAACGTTGTTGCAACAATGGGTACACAGTTGTCAGATGAACATAT
TACTTTTATACGAAAGTTAACATCAAATATAACATTAATGTTTGATGGGGATTTTGCGGGTAGTGAAG
CAACACTTAAAACAGGTCAAAATTTGTTACAGCAAGGGCTAAATGTATTTGTTATACAATTGCCATCA
GGCATGGATCCGGATGAATACATTGGTAAGTATGGCAACGATGCATTTACTGCTTTTGTAAAAAATGA
CAAAAAGTCATTTGCACATTATAAAGTGAGTATATTAAGATGAAATTGCACATAATGACCTTTTCAT
ATGAACGTTATTTGAAAGAACTAAGTCATGATATTTTCGCTTATGAAATCATCGATTTTGCACAAAAG
GCTTTAAATGATGTTGCACCATTTTTCAATGTTAGTCCTGAGCAATTAGCTAACGAAATACAATTCAA
TCAAGCACCAGCCAATTATTATCCAGAAGATGAGTATGGCGGTTACATTGAACCTGAGCCAATTGGTA
TGGCACAATTTGACAATTTGAGCCGTCAAGAAAAAGCGGAGCGAGCATTTTTAAAACATTTAATGAGA
GATAAAGATACATTTTTTAAATTATTATGAAAGTGTTGATAAGGATAACTTCACAAATCAGCATTTTAA
ATATGTATTGCAAGTCTTACATGATTTTTTATGCGGAAAATGATCAATATAATATCAGTGATGCTGTGC
AGTATGTTAATTCAAATGAGTTGAGAGAAACACTAATTAGCTTAGAACAATATAATTTGAATGACGAA
CCATATGAAAATGAAATTGATGATTATGTCAATGTTATTAATGAAAAAGGACAAGAAACAATTGAGTC
ATTGAATCATAAATTAAGGGAAGCTACAAGGATTGGCGATGTAGAATTACAAAAATACTATTTACAGC
AAATTGTTGCTAAGAATAAAGAACGCATGTAG

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.

Sheet 2 of 24

SEQ ID NO: 2

>STAAU_R009 amino acid sequence : 599

LRIDQSIINEIKDKTDILDVSEYVKLEKRGRNYIGLCPFHDEKTPSFTVSEDKQICHCFGCKKGGNV
FQFTQEIKDISFVEAVKELGDRVNVAVDIEATQSNSNVQIASDDLQMIEMHELIQEFYYYALTTKTVEG
EQALTYLQERGFTDALIKERGIGFAPDSSHCHDFLQKKGYDIELAYEAGLLSRNEENFSYYDRFRNR
IMFPLKNAQGRIVGYSGRITYTGQEPKYLNSPETPIFQKRKLLYNLDKARKSIRKLDEIVLLEGFMDVI
KSDTAGLKNVATMGTQLSDEHITFIRKLTSNITLMFDGDFAGSEATLKTGQNLLQQGLNVFVIQLPS
GMDPDEYIGKYGNDAFTAFVKNDKKSFAHYKVSILKDEIAHNDLSYERYLKELSHDISLMKSSILQQK
ALNDVAPFFNVSPEQLANEIQFNQAPANYYPEDEYGGYIEPEPIGMAQFDNLSRQEKAERAFLKHLMR
DKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNELRETLLISLEQYNLNDE
PYENEIDDYVNVINEKGQETIESLNHKLREATRIGDVELQKYYLQQIVAKNKERM

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 3 of 24

FIGURE 2

SEQ ID NO: 3

>96ORF078 nucleotide sequence

ATGAATATAATGCAATTCAAAAGCTTATTGAAATCGATGTATGAAGAGACAAAGCAAAGC
GACCCGATTGTAGCAAATGTATATATCGAGACTGGTTGGGCGGTCAATAGATTGTTGGAC
AATAACGAGTTATCGCCTTTCGATGATTACGACAGAGTTGAAAAGAAAATCATGAATGAA
ATCAACTGGAAGAAAACACACATTAAGGAGTGTTAA

SEQ ID NO: 4

>96ORF078 amino acid sequence 96_NT|10148-10363|

MNIMQFKSLLKSMYEETKQSDPIVANVYIETGWAVNRLLDNNELSPFDDYDRVEKKIMNE
INWKKTHIKEC

FIGURE 3

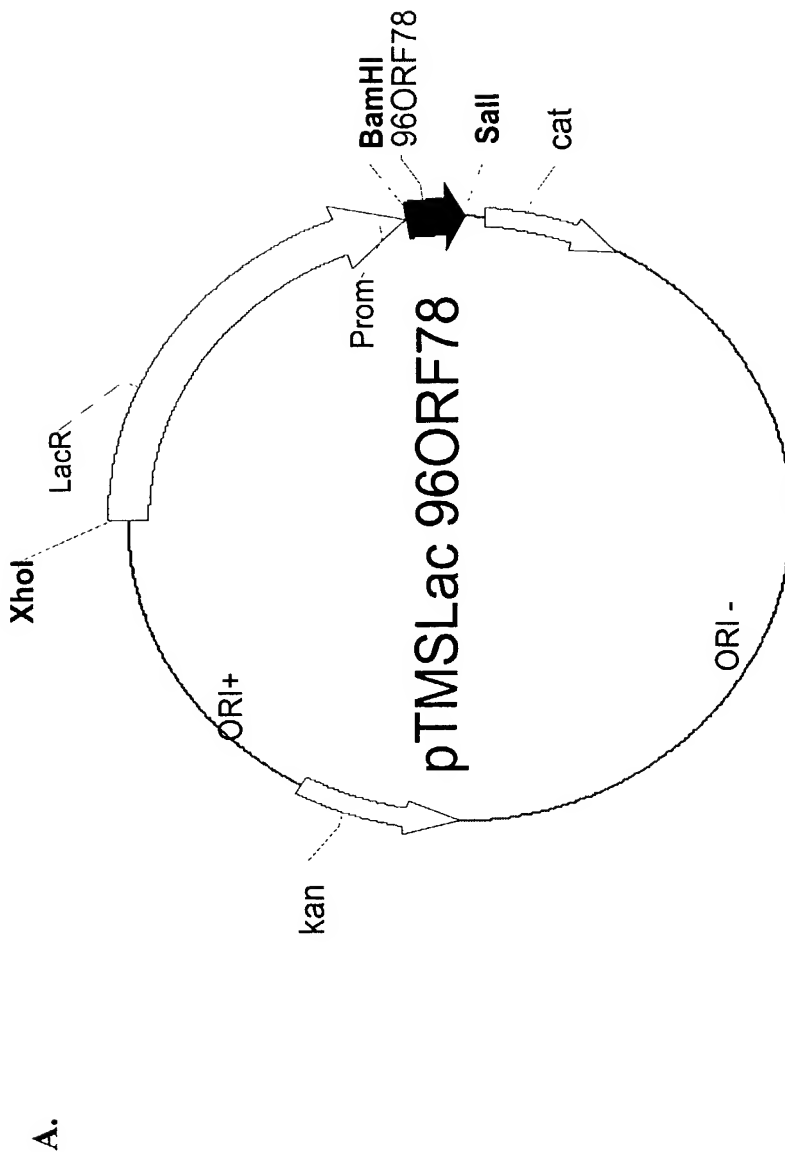
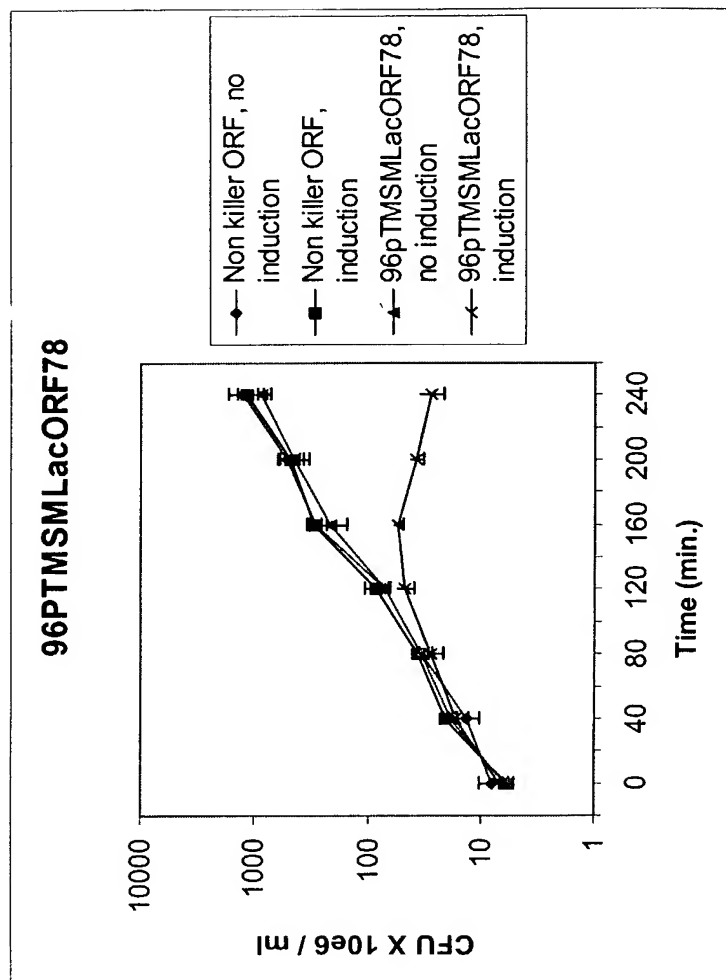


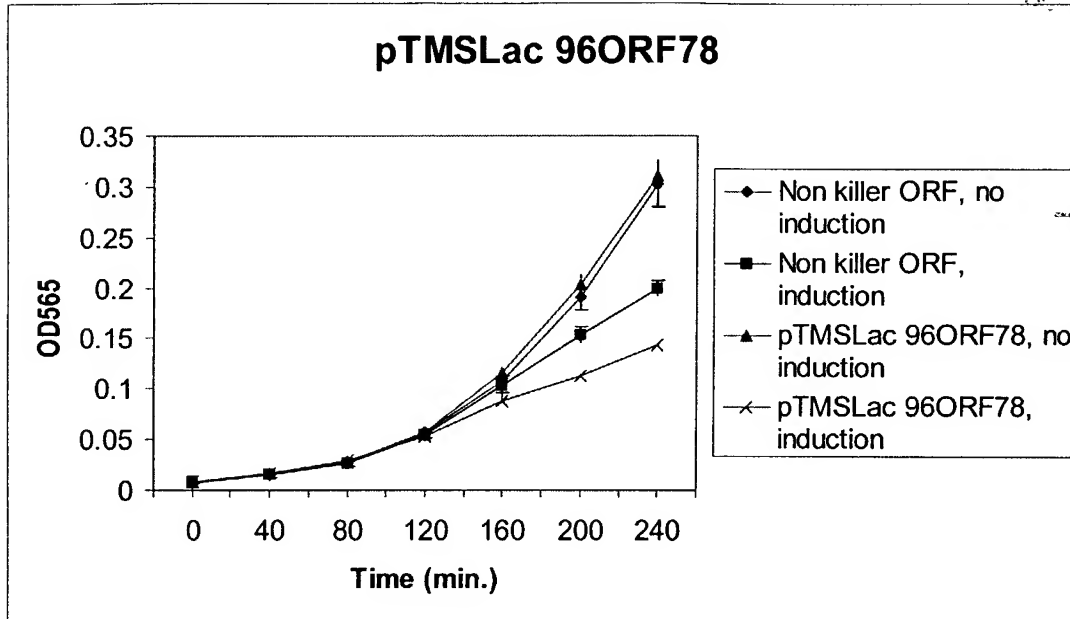
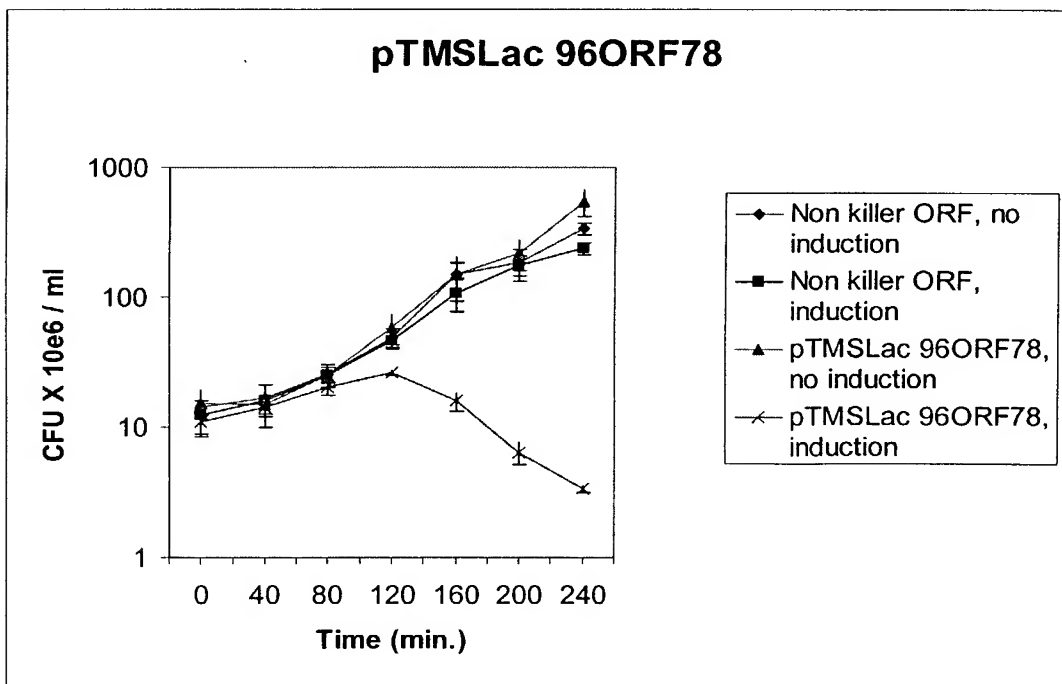
FIGURE 3 B.



Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.

Sheet 6 of 24

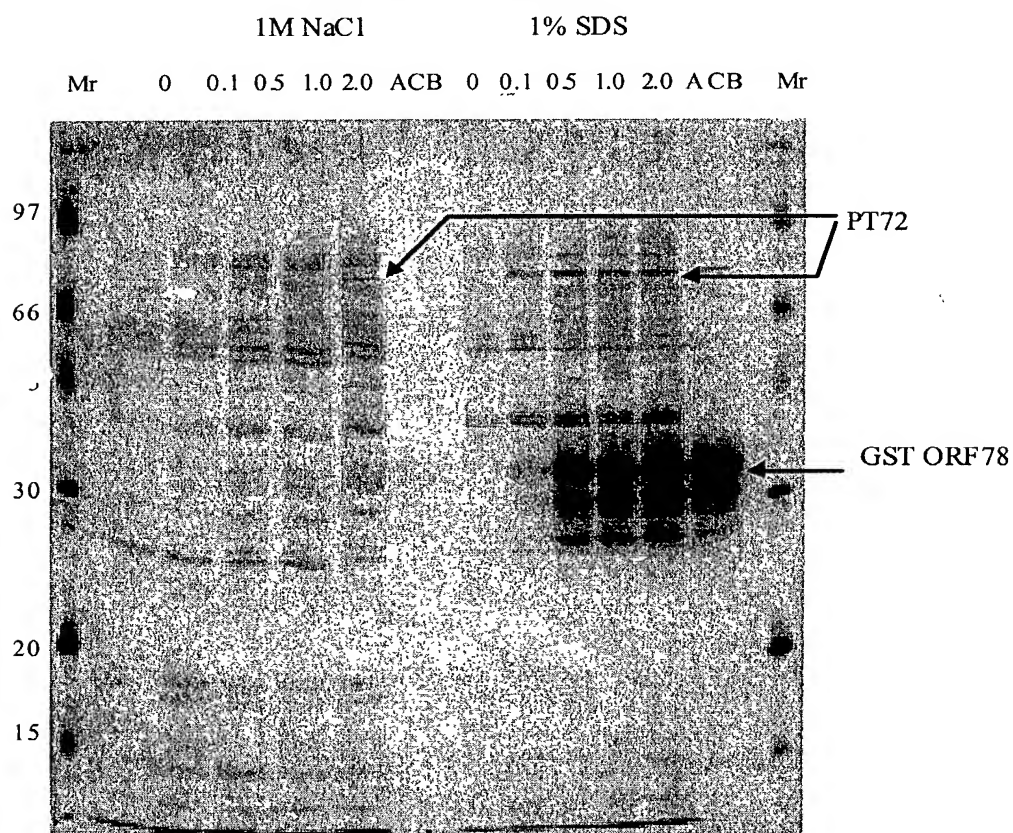
FIGURE 3 C.**D.**

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 7 of 24

FIGURE 4

A. GST/96ORF78

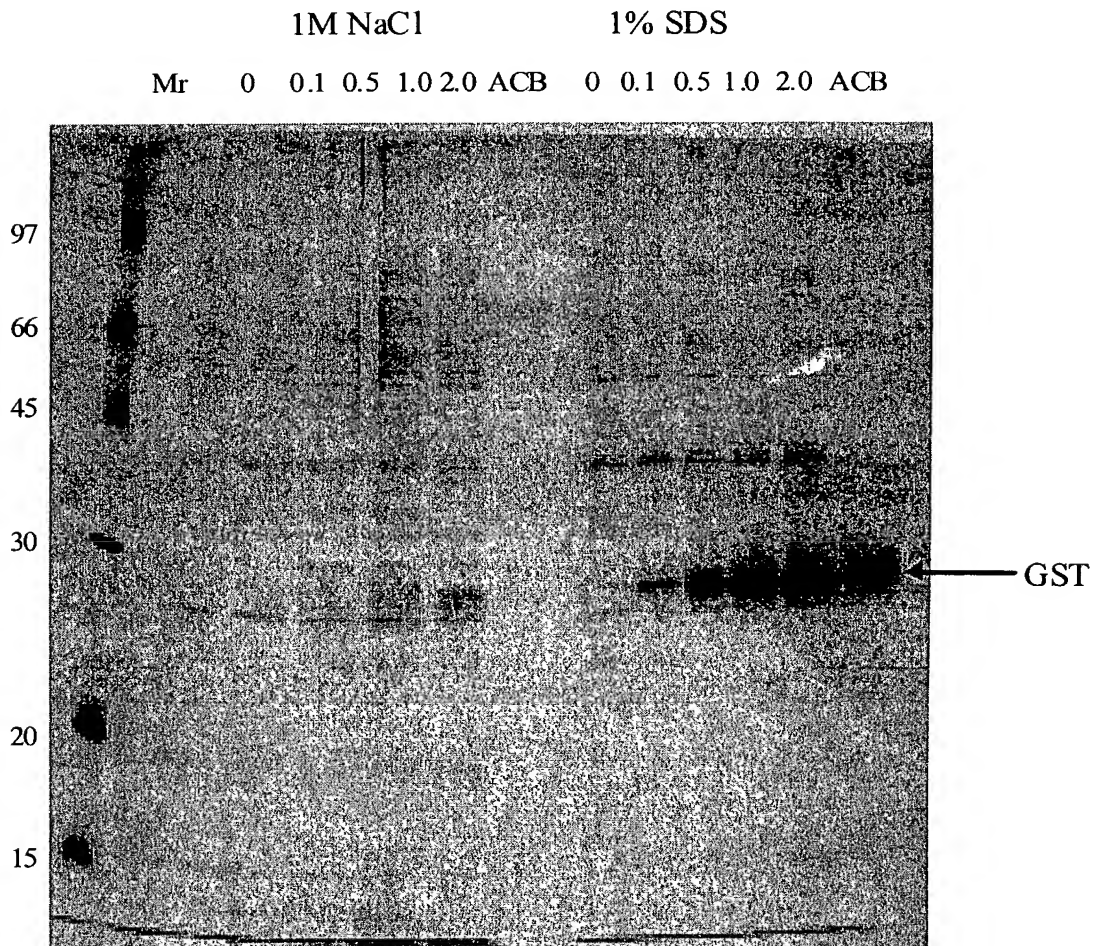


Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 8 of 24

FIGURE 4

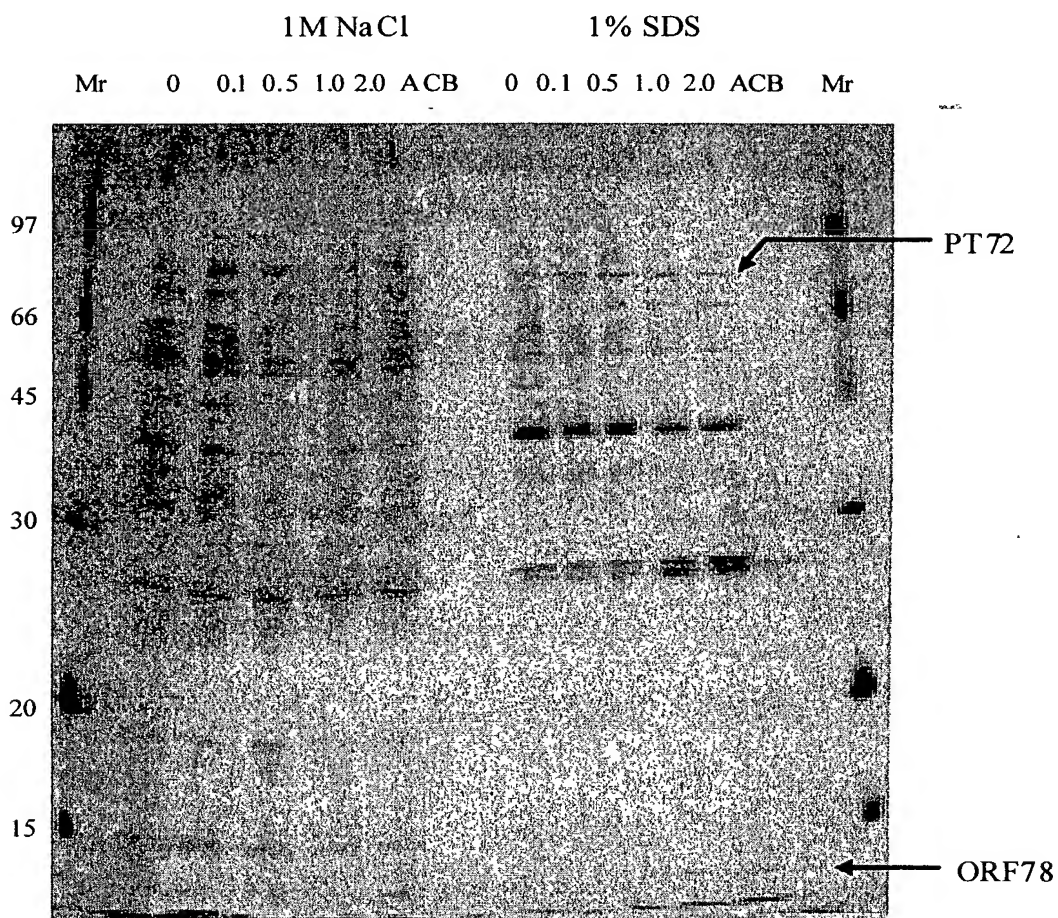
B. GST



Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 9 of 24

FIGURE 5 96ORF78 (GST removed)

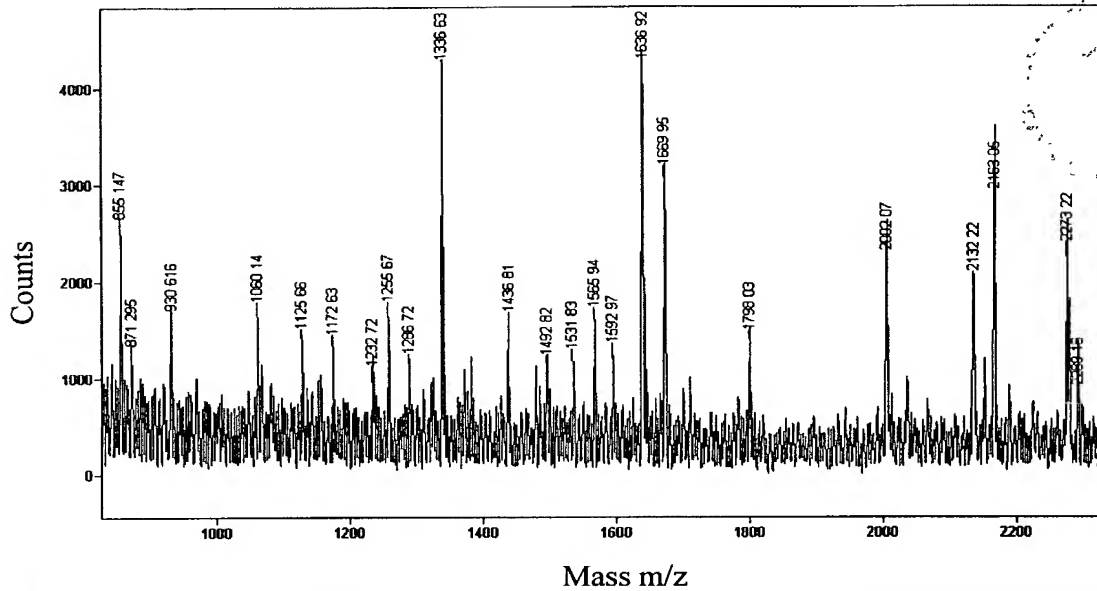


Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.

Sheet 10 of 24

FIGURE 6



Details for rank 1 candidate in search 20000915095311-0166-127000000001

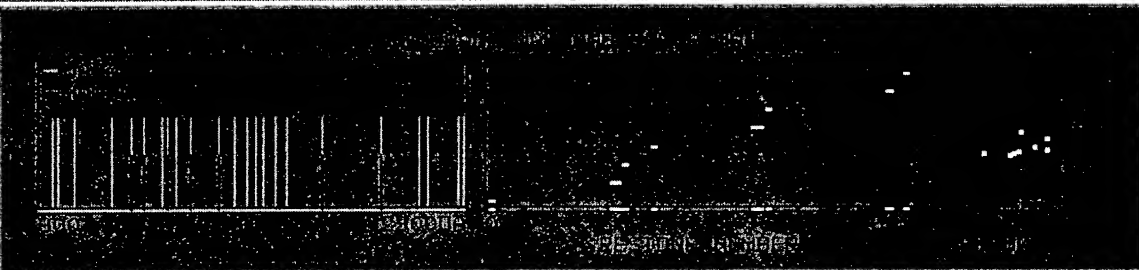
ouContig286- 82020 to 83822 (Frame 3-60) aa

Sample ID: or178.72 [Pass:0]

Measured peptides: 23

Matched peptides: 7

Min. sequence coverage: 14%



Measured Avg/ Computed Error Residues Missed

Mass(M) Mono Mass (Da) Start To Cut Peptide sequence

1124.649	M	1124.622	0.027	588	596	0	YYLQIVAK
1171.623	M	1171.590	0.033	394	403	0	ELSHDISLMK
1171.623	M	1171.644	-0.021	5	14	0	IDQSIINEIK
1254.661	M	1254.620	0.041	563	573	0	GOETIESLNHK
1335.627	M	1335.536	0.091	193	202	0	NEENFSYYDR
1435.802	M	1435.734	0.068	233	244	0	YLNSPETPIFOK
1797.019	M	1796.930	0.089	177	192	1	KGYDIELAYEAGLLSR
2001.060	M	2001.016	0.044	374	390	1	VSILKDELAHNDLSYER

Unmatched Monoisotopic Masses:

855.147 871.295 930.616 1060.140 1232.717 1286.716 1492.822 1531.830 1565.943 1592.967
1636.921 1669.948 2132.217 2163.048 2273.222 2289.164

FIGURE 7A

A. Pfam HMM search results

Model	Seq-from	Seq-to	HMM-from	HMM-to	Score	E-value
zf-CHC2	3	100	1	98	198.4	1.1e-55
Toprim	260	339	1	151	71.9	1.3e-17

Alignments of top-scoring domains:

zf-CHC2:
domain 1 of 1, from 3 to 100: score 198.4, E = 1.1e-55
*->ipeesIdelknriDIVdvisYVklKkGrnYkgLCPFHdEKTSPFs
i++++I+e+k+++DI d++seYVkl+K+GrnY+gLCPFHdEKTSPF+
gi|133988 3 IDQSIINEIKDKTDILDVSEYVVKLEKGRNYIGLCPFHdEKTSPFT 49

VspeKqfYhCFGCGagGdaIkFlmkyeklsFvEAvekLAdragidlpyek
Vs +Kq+ hCFGc++gG+++ F +++++sFvEAv++L dr+++ ++e
gi|133988 50 VSEDKQICHCFGCKKGGNVFQFTQEIKDISFVEAVKELGDRNVAVDIEA 99

g<-*
+
gi|133988 100 T 100

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Toprim: domain 1 of 1, from 260 to 339: score 71.9, E = 1.3e-17

*->kvliiVEgpsdakalakgkpskrkiyelpgkdggnvvaslGhlv

+++++ Eg++d+++ a+ +nvva++G+

gi|133988 260 DEIVLLEGFMVDVIKSDTAGL-----KNVATMTGTQ- 289

dLptpegyddykwllwlpivdvkkgfepyqiefdqckscskidlkkqeql

l+++e++

gi|133988 290 -----LSDEHI 295

kllkklakkadevilatDpDreGeaiawkllellkpygpveleddkvvrr

++kl+++ +l++D+D +G ++++k +l+ +q +v++

gi|133988 296 TFIRKLTSN---ITLMFDGDFAGSEATLKTQNLLQQCL-----NVFV 335

iflp<--*

i+lp

gi|133988 336 IQLP 339

10025222.060302

FIGURE 7B

B. Optimal global alignment of amino acid sequences

Sequence 1 sp|O05338|PRIM_STAAU DNA PRIMASE (EC 2.7.7.-) - S. aureus. (572 letters)
Sequence 2 STAAU_R009 STAAU_R009_NT|1-1800| (599 letters)

Identical: 560/605 (92%), Similar: 564/605 (93%), Gap: 39/605 (6%)
seq1 1 M-----IGLCPFHDEKTPSFTVSEDKQICHCF 27
: |||||||
seq2 1 LRIDQSIINEIKDKTDILDVSEYVKLEKGRNYIGLCPFHDEKTPSFTVSEDKQICHCF 60

seq1 28 GCKKGGNVFQFTQEIKDISFVEAVKELGDRNVAVDIEATQSNQVQIASDDDLQMIEMHE 87
|||||
seq2 61 GCKKGGNVFQFTQEIKDISFVEAVKELGDRNVAVDIEATQSNQVQIASDDDLQMIEMHE 120

seq1 88 LIQEFYYVALTKTVEGEQALTYLQERGFDTALIKERGIGFAPDSSHCHDFLQKKGYDIE 147
|||||
seq2 121 LIQEFYYVALTKTVEGEQALTYLQERGFDTALIKERGIGFAPDSSHCHDFLQKKGYDIE 180

seq1 148 LAYEAGLLSRNEENFSYDRFRNRIMFPLKNAQGRIVGYSGRITYTGQEPKYLNSPETPIF 207
|||||
seq2 181 LAYEAGLLSRNEENFSYDRFRNRIMFPLKNAQGRIVGYSGRITYTGQEPKYLNSPETPIF 240

seq1 208 QKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTLSDHEHITFIRK 267
|||||
seq2 241 QKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTLSDHEHITFIRK 300

seq1 268 LTSNITLMFDGDFAGSEATLKTGQHLLQQGLNVFVIQLPSGMDPDEYIGKYGNDAFTTFV 327
|||||:|||||
seq2 301 LTSNITLMFDGDFAGSEATLKTGQNLLQQGLNVFVIQLPSGMDPDEYIGKYGNDAFTAFV 360

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Sheet 14 of 24

[illegible]

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN
Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 16 of 24

seq2	358	AGEAGCRRPLVAFKMAYL	---	RGKNLQHEGERLRYIDEALREIGKLSSPVEQDY	YLRQL	414
seq1	414	APFFNVSPQLANEIQFNQAPANYYPEDEYGGYIEPEPIGMAQFDNLSRQEKAE	FLKH			473
seq2	415	AEFSLSLSALHEQLSRQRERTKPREAPDGETARP	----	MLAKLLLPAFQNAERL	LILLAH	470
seq1	474	LMRDKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNEL	RETL			533
seq2	471	MMRSRDVALVQERIG-GRFNIEEHRALAAIYAFYEEGHEADPGALISRI-PGELQPLA				528
seq1	534	ISLEQYNLNDEPYENEIDDYV-NVINEKGQETIESLNHKLREATRIGDVELQKY	YLQQIV			592
seq2	529	SDVSLLLIADDVSEQELEDYIRHVLNRPKWLMLKVKEQEKTEAERRKDFLTAARIAKEMI				588
seq1	593	AKNK--ERM	599			
seq2	589	EMKKMLSSS	597			

10005222.060302

Sheet 18 of 24

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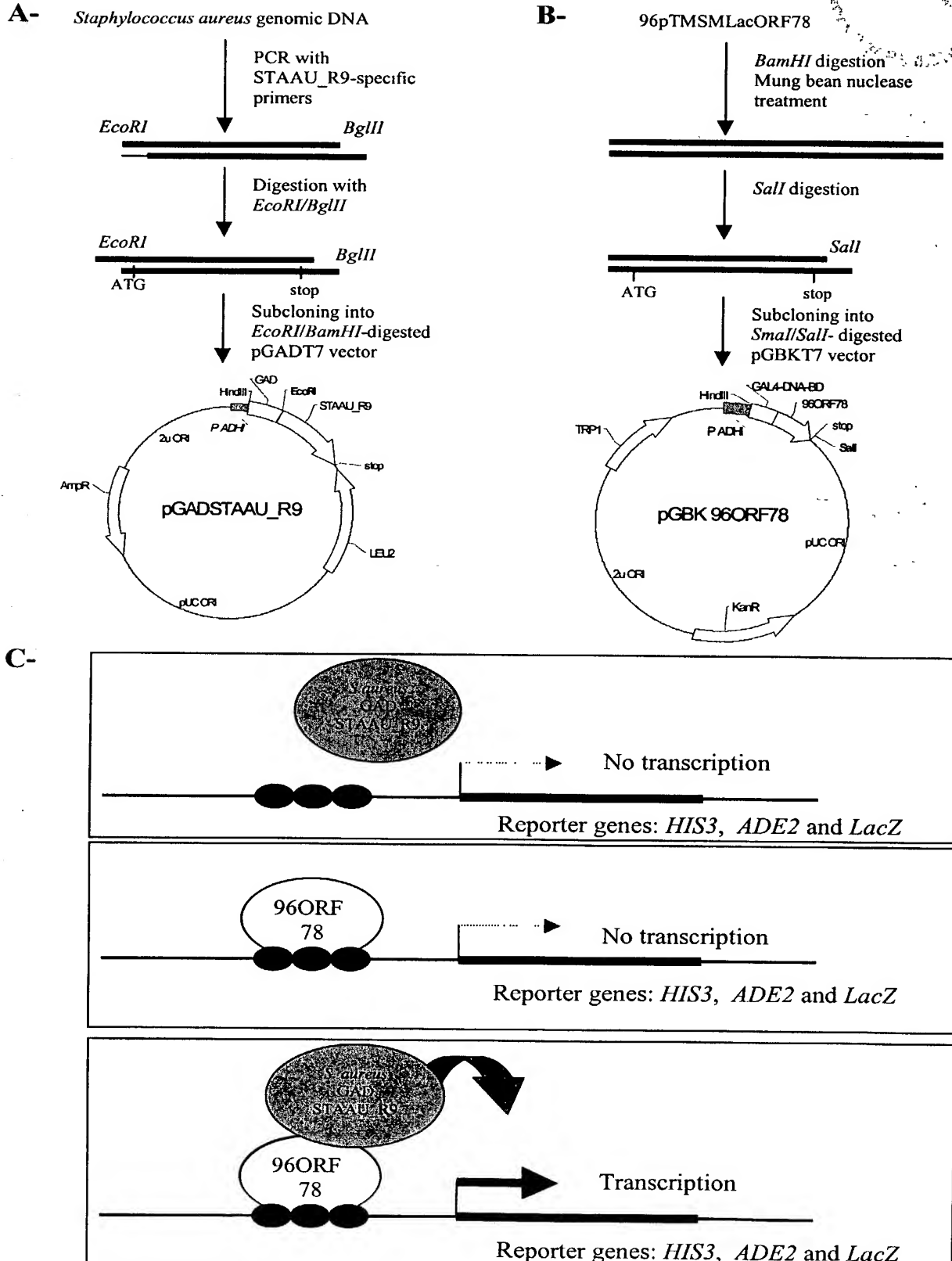
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AUREUS GENE AND ITS ENCODED PROTEIN
Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 20 of 24

seq1	413	VAPFFNVSPEQLANEIQF--NQAPANNYPPEDEYGGYIEPEPIGMAQFDNLSRQEKAEAF	470
	:	: : : : : :	:
seq2	407	LRIYLR---QELGNKLGILDDSQLERLMPKAAESGVS RVP-----QLKRRTMRIL	454
	:	: : : : : : :	:
seq1	471	LKHLMRDKD--TFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNE	528
	:	: : : : : : :	:
seq2	455	IGLLVQNPELATLVPPLNLDENKLPGLG---LFREL VNTCLS QPGLTTGQLLEHYRGTN	511
	:	: : : : : : :	:
seq1	529	LRETLISLEQY-----NLNDEPYENEIDDYVNVINEKGQETIESLNHKLREATR--IGD	580
	:	: : : : : : :	:
seq2	512	NAATLEKLSMWDDIADKNIAEQFTTDSLNMHMFDSLLELRQEELIA-----RERTHGLSNE	566
	:	: : : : : : :	:
seq1	581	VELQKYYLQQIVAKNKERM	599
	:	: : : : : : :	:
seq2	567	ERLELWTLNQELAKK----	581
	:	: : : : : : :	:

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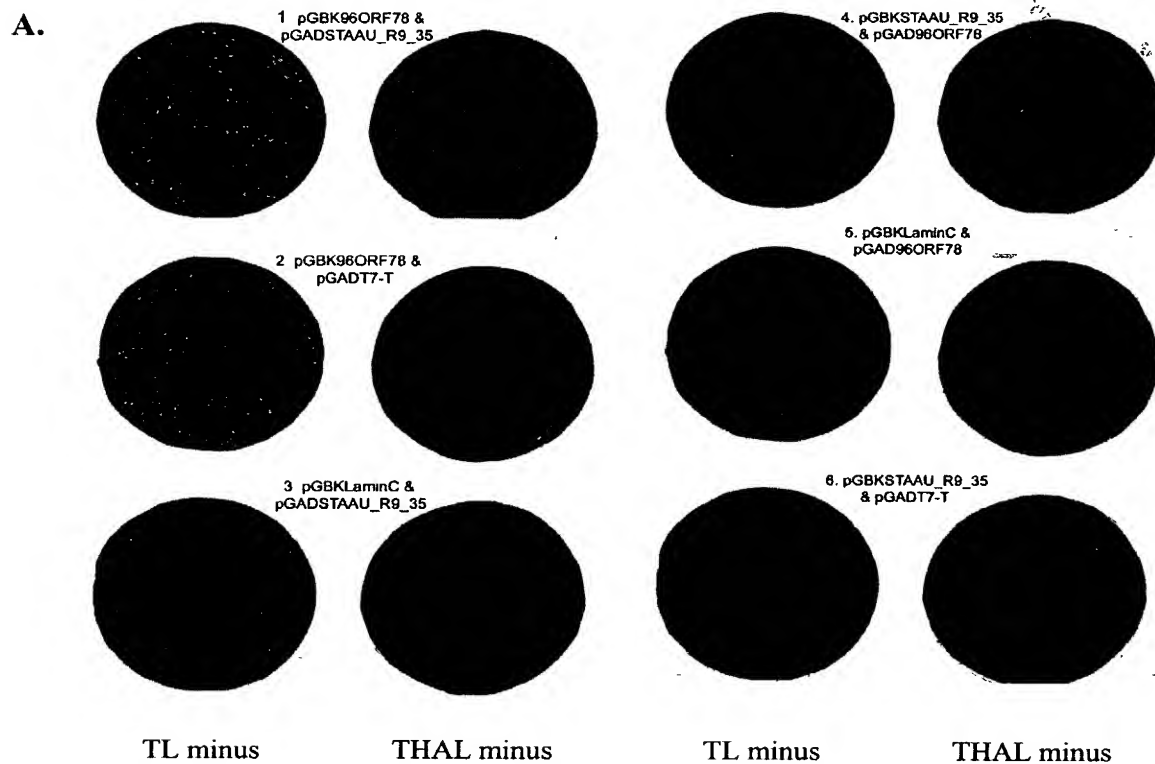
FIGURE 8



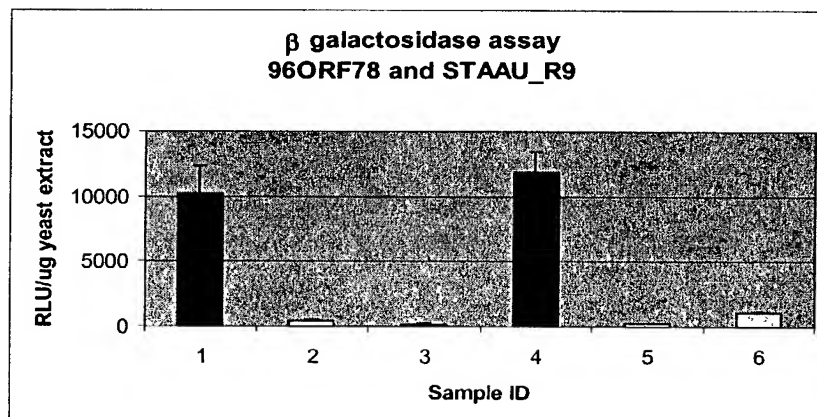
Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
Sheet 22 of 24

FIGURE 9



B.



Sample	Yeast co-transformants		RLU (avg)	SD
	Plasmid 1	Plasmid 2		
1	pGBK 96ORF78	pGADSTAAU_R9_35	10,250	2,080
2	pGBK 96ORF78	pGADT7-T	475	38
3	pGBK Lamin C	pGADSTAAU_R9_35	224	10
4	pGBKSTAAU_R9_35	pGAD 96ORF78	11,935	1,477
5	pGBK Lamin C	pGAD 96ORF78	243	12
6	pGBKSTAAU_R9_35	pGADT7-T	1,121	37

FIGURE 10

A. Fragments of STAAU_R9

B.

Interaction with
96ORF78

1	599	Yes
35	599	Yes
35	342	No
229	402	No
229	599	Yes
380	599	Yes
380	449	No
380	490	No
380	530	No
380	561	No
449	599	Yes
490	599	Yes
530	599	Yes
561	599	Yes

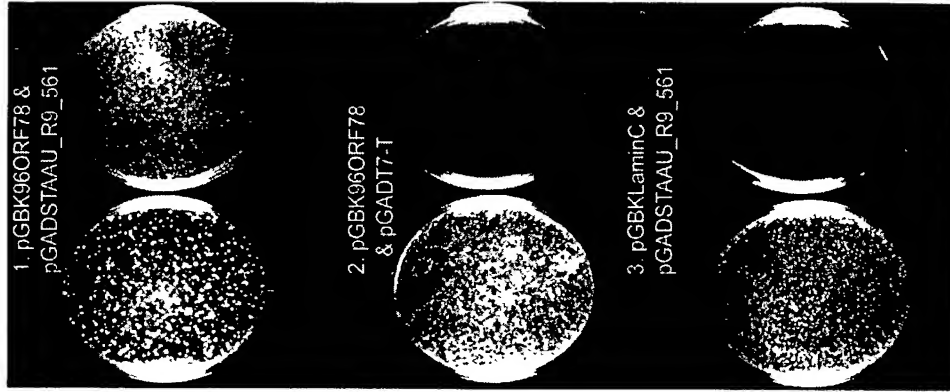
SEQ ID NO: 6 STAAU_R9_561-599
AMINO ACID SEQUENCE

QGETIESLNHLKREATRIGDVELQKYLLQIVAKNKERM

SEQ ID NO: 5 STAAU_R9_1683-1800
NUCLEIC ACID SEQUENCE

GGACAAGAAACAATTGAGTCATTGAATCATATAAATTAAAGGGAAGCTACAAGGATTGGCGATGTGA
GAATTACAAAATACTATTACAGCAAATTGTGCTAAGAATAAAGAACCGCATGTAG

C.



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Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN

Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.

Sheet 24 of 24

FIGURE 11

A.

	Primer name	Primer sequence	Restriction site
SEQ ID NO: 8	R9_5E3	5'-ccggaattcTTGCGAATAGATCAATCG-3'	EcoRI
SEQ ID NO: 9	R9_3BG	5'-ggaagatctCTACATGCGTTCTTTATTC-3'	BglII
SEQ ID NO: 10	R9_5E	5'-ccggaattcATGATAGGTTTGTGTCCT-3'	EcoRI
SEQ ID NO: 11	R9_5E1	5'-ccggaattcCCAAAATACCTAAATAGTCC-3'	EcoRI
SEQ ID NO: 12	R9_5E2	5'-ccggaattcGCACATAATGACCTTTCA-3'	EcoRI
SEQ ID NO: 13	R9_342R	5'-cgcgatccATGCCTGATGGCAATTG-3'	BamHI
SEQ ID NO: 14	R9_402R	5'-ccatcgatGATTCATAAGCGAAATATC-3'	ClaI
SEQ ID NO: 15	R9_449F	5'-ccggaattcCCTGAGCCAATTGGTATGGC-3'	EcoRI
SEQ ID NO: 16	R9_449R	5'-cgcgatccctaAGGTTCAATGTAACCGCC-3'	BamHI
SEQ ID NO: 17	R9_490F	5'-ccggaattcAAGGATAACTTCACAAATCAG-3'	EcoRI
SEQ ID NO: 18	R9_490R	5'-cgcgatccctaCTTATCAACACTTTCATAATA-3'	BamHI
SEQ ID NO: 19	R9_530F	5'-ccggaattcAGAGAAACACTAATTAGCTTA-3'	EcoRI
SEQ ID NO: 20	R9_530R	5'-cgcgatccctaTCTCAACTCATTTGAATTAAC-3'	BamHI
SEQ ID NO: 21	R9_561F	5'-ccggaattcGGACAAGAAACAATTGAGTC-3'	EcoRI
SEQ ID NO: 7	R9_561R	5'-cgcgatccctaTCCTTTTTCATTAATAACATTG-3'	BamHI

B.

Cloning of SEQ ID NO: 2 amino acid fragments	Sense primer	Antisense primer
1-599	R9_5E3	R9_3BG
35-599	R9_5E	R9_3BG
35-342	R9_5E	R9_342R
229-402	R9_5E1	R9_402R
229-599	R9_5E1	R9_3BG
380-599	R9_5E2	R9_3BG
380-449	R9_5E2	R9_449R
380-490	R9_5E2	R9_490R
380-530	R9_5E2	R9_530R
380-561	R9_5E2	R9_561R
449-599	R9_449F	R9_3BG
490-599	R9_490F	R9_3BG
530-599	R9_530F	R9_3BG
561-599	R9_561F	R9_3BG